

FIG. 1

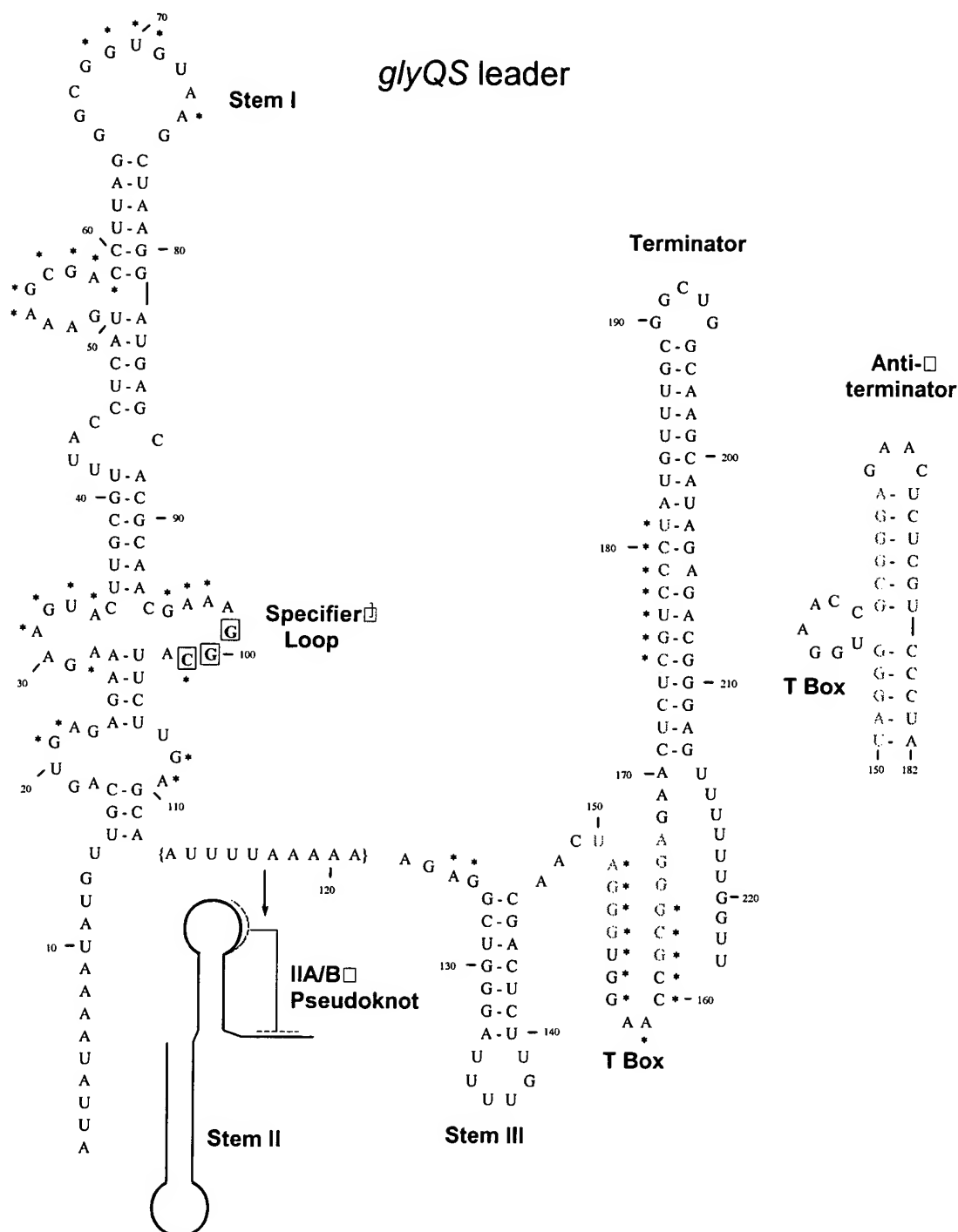


FIG. 2

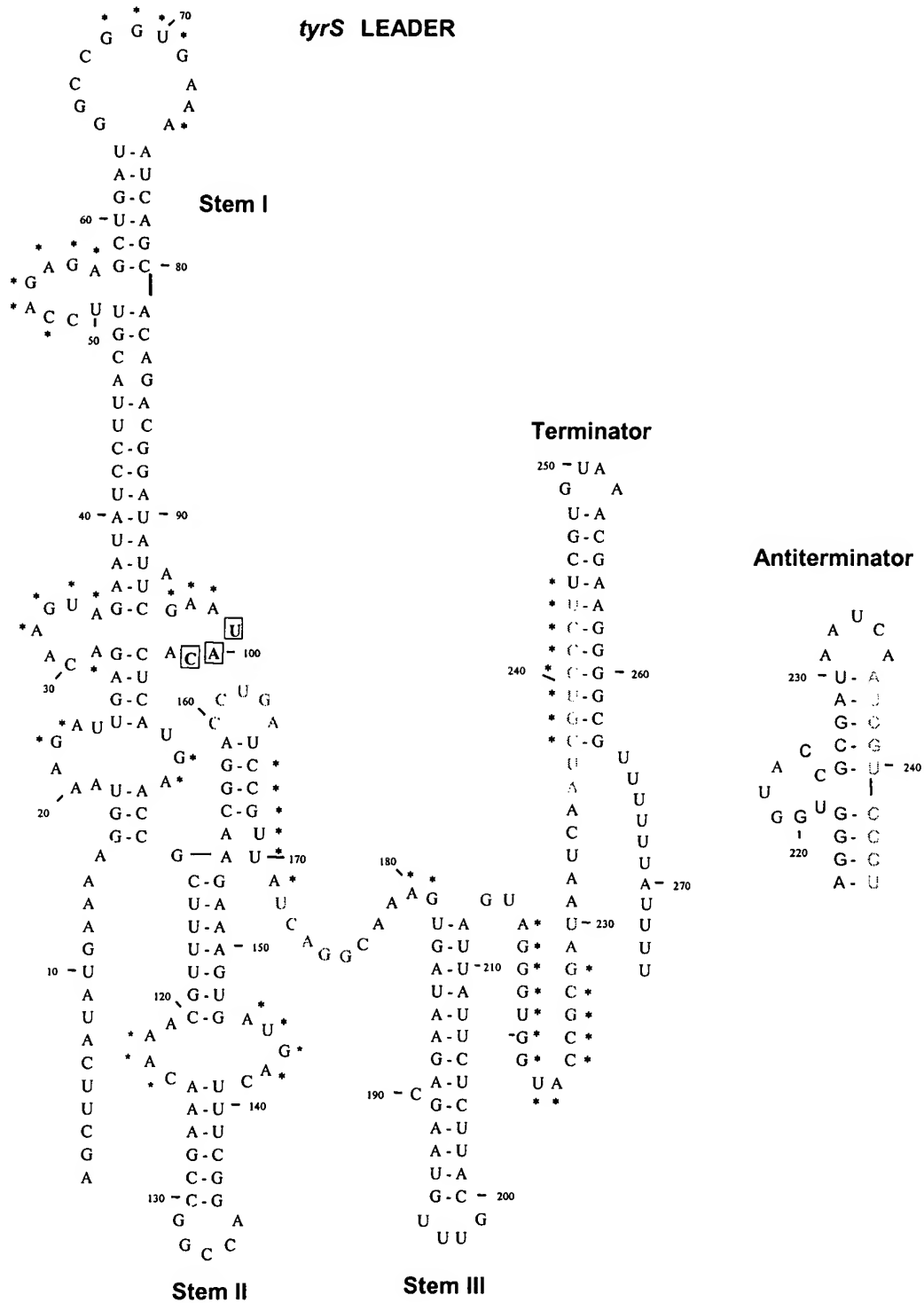


FIG. 3: Panel A

B. sub tyrs AGCTTCATATGAAAAGGTAAAGATCGAGA---CAAGTAGA-ATATCCTT---TACGT---TCCAGAGA--GCT---GAT
 B. ant glys ATTATTAATATAAGTAGCGATGACGGAAT-TATAAGTACTTGG---ACA---AAAAGCGA--TTC---AGG
 B. cer glys ATTATTAATATAAGTAGCGATGACGGAAT-TATAAGTACTTGG---ACA---AAAAGCGA--TTC---AGG
 B. hal glys AATGTTATATTTCAATGCTATGACGGAAT-ACAGTACTTGATTCCT---TTTACAT---AAAAGCGA--ACC---TAG
 B. ste glys AAATCATATATGGATCGGATGAGATC--AATAGTAGTTAAACCTC---TCTTC---CCAAAGCGA--GCC---GGG
 B. sub glys TATTTATTAATATGTTGACGTGAGAGAA--GAAGTACTTGGCTTTA---CCTCAT---GAAAGCGA--CCT---TAG
 C. ace glys ATAAATTAATATCTATACAAATGACAAAGAT---AGAAATGTAATTT---CCT---CAAAAGAGA--GGC---TGT
 C. hyd glys AATTAATAATGGATGGCAGTGAACCGA---GGAGTAGCTGTGATTT---CCCT---TAAAGAGA--GCC---GGG
 D. rad glys -----GGAGGCGTTGAACCGA---GGAGTACCG-CAAAGA---GCCCGA---ACGAGCGA--GCC---TGA
 E. fae glys GAGAAGTTAAATAGCTACGAAGAAAGAA--GAAGTAAAGAAACCC---TCTGTT---AAGCGAA-TCT---GG
 L. lac glys GCCTTGACAAAATGGAAAATAGAGCTAG---AATTATATA---GGT---AGCGA--CTC---GAA
 L. mon glys ATATCAACTAATAGTAGCTGTTAGGAA---ATAGTAACAAAAGCT---CT-AT---TTTACCGA--GTC---CGG
 S. aur glys ATGTCACAAACACATTAATTTACTTGC--TTTAAATAATCTATCAA-----TT---*GTACAGCGA--GTT---AAG
 S. equ glys TTTGCGATAAACTAACCAATAG-GAAAGA-----AAATAGCAGG---TTTCTGATC---TAAAGCGA--GCT---CGG
 S. mut glys -----GTTAAGAAGA-----GAGT---TTTGT---GGCGTTTC---TGCAGCGA--ACC---TGA
 S. pne glys ATTTTGGATATAATAGTCAGCAGGAAAGA-----AAGTCTT-AT-----GGCGTTCT---TCAAGCGA--GCT---TGG
 S. pyo glys GCTTTATGCTAAACTAGACTCTAGAAAA-----AGGATCAAG---TATCTTATC-----TAAAGCGA--GTT---CGG

FIG. 3: Panel B

B. sub tyrs ---GGCCGGTGAAA--ATC---AGCACAGA-----CGATATATC--GAA---TACA--CTCATGAACC-----
 B. ant glys ---GATAGTGAAG-CCT---GAAGCC-----GCAAG--GAA-ACGGCA--GTCTCGAGC-----
 B. cer glys ---GATAGTGAAG-CCT---GAAGCC-----GCAAG--GAA-ACGGCA--GTCTCGAGC-----
 B. hal glys ---GATGGTGAGAG-CTA---GGGATGTAAA-----CATCAAG--GAA---GGCA--CTCTTGAGCA-TGAA---
 B. ste glys ---GACGGTGAAG-CCC---GGCGAAGA-----TGTTAAT--GAA-ACGGCA--GTCTGGAGCG-----
 B. sub glys ---GGCGGTGAAG-CTA---AGGATGAG-----CAGCAAC--GAA-AGGCA--TTCTTGAGCAA-----
 C. ace glys ---GGAGGGTGTA--ACG---GTCAAG-----AAATTCAGT--AGT---GGAG--TCCTTCGAGTA-----
 C. hyd glys ---GGCTGGTGTA--ACC---GGTAGGG-----A-TAAACGGT--GAA---GGCG--CCGCGGAGCCG-----
 D. rad glys ---GACGGTGAGAG-TCA---GGCAGGGTGA-----GGCGGACGG--GAA-AGGCA--GGGCGAGCG-----
 E. fae glys ---GAGAGTGGAG-CC---AGAAACA-----CGGAACCTTT--GAA-AGGCA--CTTGGAGTACG-----
 L. lac glys ---GATAGTGAAG-TTC---GAGAAC-----AATAA--T-----GGCT--TAACT-----
 L. mon glys ---GTTTGGTGAG-CCG---GATATTA-----ACITTTTGT--GAA---GGCG--TTCTGGAGTA-----
 S. aur glys ---GATAGTGAAG-CTT---AACAA-----T---AAGATT-----GGC--GCAACGAATC-----
 S. equ glys ---GGCTGGTGAGAG-CCG---AGTGAT-GG-T---ACTGCTGGTT-----GGCG--CTCTCTTA-----
 S. mut glys ---GAGAGTGAAG-TCA---GGTGAACAAA-----ATAAGGACT-----GGCA--CTTCT-----
 S. pne glys ---GATAGTGGAG-CCA---AGTAGGGCAA-----ATAAGGGCT-----GGCG--CTTCT-----
 S. pyo glys ---GGTAGTGAAG-CCG---AATGGTAGGA-----CTGCAGATT-----GGCG--CTTCTGTT-----

FIG. 3: Panel C

B. sub <u>tyrS</u>	GCT	TTTGC	AAAC	AAAGCC	GGCCA	GGCTTT	CAGTA	GTGAA	AGA
B. ant <u>glyS</u>					AATACGTGATAA				
B. cer <u>glyS</u>					AATACGTGATAA				
B. hal <u>glyS</u>					CGATGAAAAGAA				
B. ste <u>glyS</u>					GAAATGGCAAAA				
B. sub <u>glyS</u>									
C. ace <u>glyS</u>	TTT	TT		AAAA			GA		AAA
C. hyd <u>glyS</u>	GCA		GGAGGAAA	CCC	CAAG	GGG	AGTAAAGCC		TGC
D. rad <u>glyS</u>									
E. fae <u>glyS</u>									
L. lac <u>glyS</u>			AAACTGTAATGAAC		ACAAAT		AAAGTAAAAA		
L. mon <u>glyS</u>					CAGCGAAATCAA				
S. aur <u>glyS</u>					ATTTTAAAAATAAA				
S. equ <u>glyS</u>					AAGAGTAGGCTCA				
S. mut <u>glyS</u>	CTT		GGCT		AAT		AGCC		AAG
S. pne <u>glyS</u>									
S. pyo <u>glyS</u>	GGG	CAGTGT	GATTAAG		TATA		TTT	GTCAATAT	CCC

FIG. 3: Panel D

B. sub <u>tyrS</u>	ACGGA				CCTGA	TCCGTATCAGG	
B. ant <u>glyS</u>							
B. cer <u>glyS</u>							
B. hal <u>glyS</u>							
B. ste <u>glyS</u>							
B. sub <u>glyS</u>							
C. ace <u>glyS</u>	GC		AGG		GCTATTGCC		
C. hyd <u>glyS</u>							
D. rad <u>glyS</u>							
E. fae <u>glyS</u>							
L. lac <u>glyS</u>							
L. mon <u>glyS</u>							
S. aur <u>glyS</u>							
S. equ <u>glyS</u>							
S. mut <u>glyS</u>							
S. pne <u>glyS</u>							
S. pyo <u>glyS</u>							

FIG. 3: Panel E

B. sub <u>tyrS</u>	-----AAAGTGA-----TAAGACGAATGTTTG-----CATTC-TCTTA-----TTA
B. ant <u>glys</u>	-----AGTGGAT-----GCACCTTTT-----GTGT-----ATC
B. cer <u>glys</u>	-----AGTGGAT-----GCACCTTTT-----GTGT-----ATC
B. hal <u>glys</u>	-----AGTGGCC-----TATGGTGTC-----ATCATA-----GGC
B. ste <u>glys</u>	-----AGGGAT-----GCGTGATT-----TGC GC-----ATC
B. sub <u>glys</u>	-----TTTTAAAAAAGAGGCT-----GGGATTTTGT-----TCTC-----AGC
C. ace <u>glys</u>	-----AGAGTTTTCAGGTGGC-----CTTTTT-----G-----GCC
C. hyd <u>glys</u>	ACAACCGGTCTGAAAGTGCTG-----GCGAGG-----GC-----CAG
D. rad <u>glys</u>	-----ACAAACGAAGCT-----GCCGATGAACA-----CATCGG-----AAG
E. fae <u>glys</u>	-----GGTGGGA-----ATTGTTT-----TAAT-----TCC
L. lac <u>glys</u>	-----AGCGAGT-----GACTA-----C-----ACT
L. mon <u>glys</u>	-----GGTGTTT-----GTAGCTTGCTTGACATCTGTT-----TATCAA-CAAG-ATC-----AAA
S. aur <u>glys</u>	-----CTAACA-----ATCAGATAAA-----TGA-----AGT
S. equ <u>glys</u>	-----GTAGTAT-----T-TTCAAAAACAA-----TGAAGTA-----ATA
S. mut <u>glys</u>	-----AAAAAGATAC-----TATATAAA-----TGAA-----GTA
S. pne <u>glys</u>	
S. pyo <u>glys</u>	

FIG. 3: Panel F

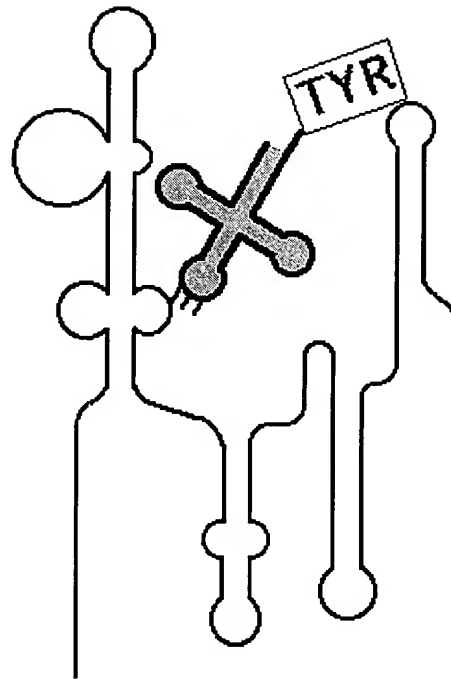
B. sub <u>tyrS</u>	-----GTAGGTGGTACCGCAT-----AATCA-----ATCGT-----CCCT-----
B. ant <u>glys</u>	-----AACTAGGTGGAACCGGGC-----AAAC-----GCTCGT-----CCCTAG-----
B. cer <u>glys</u>	-----AACTAGGTGGAACCGGGC-----AAAC-----GCTCGT-----CCCTAG-----
B. hal <u>glys</u>	-----AAATAGGTGGAACCGGGG-----TTAACT-----CTCGT-----CCCTAT-----
B. ste <u>glys</u>	-----AACTAGGTGGAACCGGGGAGC-----TAC-----GCTCTCGT-----CCCTAG-----
B. sub <u>glys</u>	-----AACTAGGTGGAACCGGGGA-----GAAC-----TCTCGT-----CCCTA-----
C. ace <u>glys</u>	-----AATAAGGTGGAACCGGGAA-----GTAA-----TTTCGT-----CCCTT-----
C. hyd <u>glys</u>	-----AACCAGGTGGAACCGGGAAG-----ATGCC-----CCTTTCGT-----CCCTGG-----
D. rad <u>glys</u>	-----AACTGGGTGGAACCGGCATG-----TCC-----CGTGGC-----CCCGG-----
E. fae <u>glys</u>	-----TAGGTGGAACCGGA-----TAATTAT-----TCGT-----CCCTA-----
L. lac <u>glys</u>	-----ATAAGGTGGAACCGTGC-----ATTT-----GCAC-----CCTTTGT-----
L. mon <u>glys</u>	-----AAATAGGTGGAACCGCGAG-----CTAACT-----CTCGT-----CCCTAT-----
S. aur <u>glys</u>	TGAAGTAATAATTAGSGTGAACCGGTT★-----TTTG-----ACGC-----CCCA-----
S. equ <u>glys</u>	-----AATAAAATTAGSGTGAACCGGTT-----TCA-----AACGC-----CCCTA-----
S. mut <u>glys</u>	-----AATTAGGTGGAACCGGTT-----TCT-----GACGC-----CCCTAG-----
S. pne <u>glys</u>	-----ATAAAATTAGGTGGAACCGGTT-----TTTG-----ACGC-----CCCTAG-----
S. pyo <u>glys</u>	

FIG. 4

	ORGANISMS WITH LEADER SEQUENCES HAVING THE T BOX PATTERN	NCBI GENOME SEQUENCE FILE NAME
5	<i>Bacillus anthracis</i>	NC_003997
	<i>Bacillus cereus</i>	NC_004722
	<i>Bacillus halodurans</i>	NC_002570
	<i>Bacillus subtilis</i>	NC_000964
10	<i>Carboxydotherrnus hydrogenoformans</i>	NC_002972
	<i>Clostridium acetobutylicum</i>	NC_003030
	<i>Clostridium botulinum</i>	NC_003223
	<i>Clostridium difficile</i>	NC_002933
	<i>Clostridium perfringens</i>	NC_003366
15	<i>Clostridium tetani</i>	NC_004557
	<i>Clostridium thermocellum</i>	NZ_AABG000000000
	<i>Corynebacterium glutamicum</i>	NC_003450
	<i>Corynebacterium diptheriae</i>	NC_002935
	<i>Deinococcus radiodurans</i>	NC_001263
20	<i>Desulfotobacterium hafniense</i>	NZ_AAAW000000000
	<i>Enterococcus faecalis</i>	NC_004668
	<i>Enterococcus faecium</i>	NZ_AAAK000000000
	<i>Geobacillus stearothermophilus</i>	NC_002926
	<i>Geobacter sulfurreducens</i>	NC_002939
25	<i>Lactococcus lactis</i>	NC_002662
	<i>Leuconostoc mesenteroides</i>	NZ_AABH000000000
	<i>Listeria innocua</i>	NC_003212
	<i>Listeria monocytogenes</i>	NC_003210
	<i>Mycobacterium leprae</i>	NC_002677
30	<i>Mycobacterium tuberculosis</i>	NC_000962
	<i>Oceanobacillus iheyensis</i>	NC_004193
	<i>Oenococcus oeni</i>	NZ_AAAAZ000000000
	<i>Ruminococcus albus</i>	NC_003373
	<i>Spiroplasma kunkelii</i>	NC_003999
35	<i>Staphylococcus aureus</i>	NC_002758
	<i>Staphylococcus epidermidis</i>	NC_004461
	<i>Streptococcus equi</i>	NC_002955
	<i>Streptococcus gordoniae</i>	NC_002979
	<i>Streptococcus mutans</i>	NC_004350
40	<i>Streptococcus pneumoniae</i>	NC_003098
	<i>Streptococcus pyogenes</i>	NC_002737
	<i>Streptomyces coelicolor</i>	NC_003888
	<i>Thermoanaerobacter tengcongensis</i>	NC_003869
	<i>Thermobifida fusca</i>	NZ_AAAQ000000000

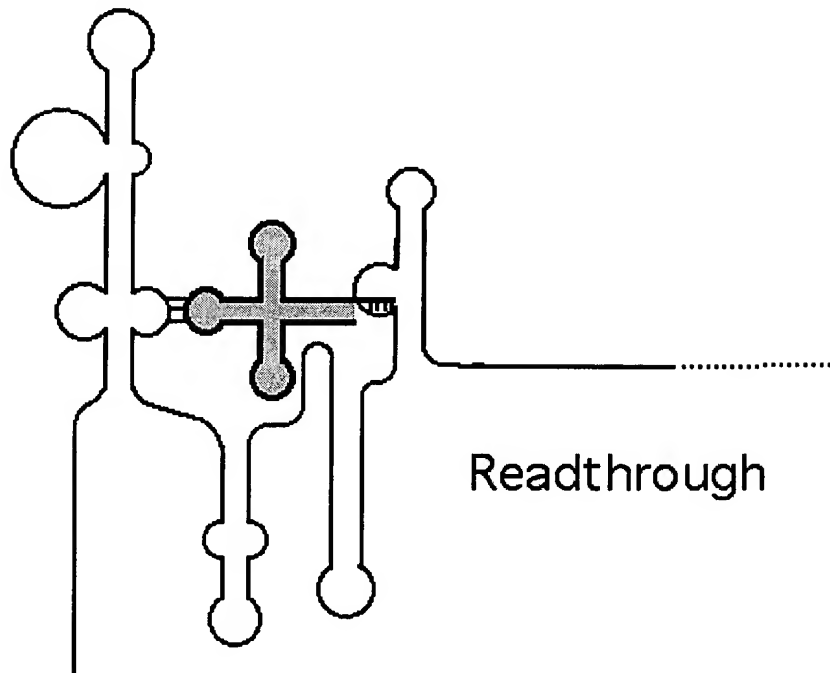
FIG. 5

High tRNA
charging



Terminate

Low tRNA
charging



Readthrough

FIG. 6

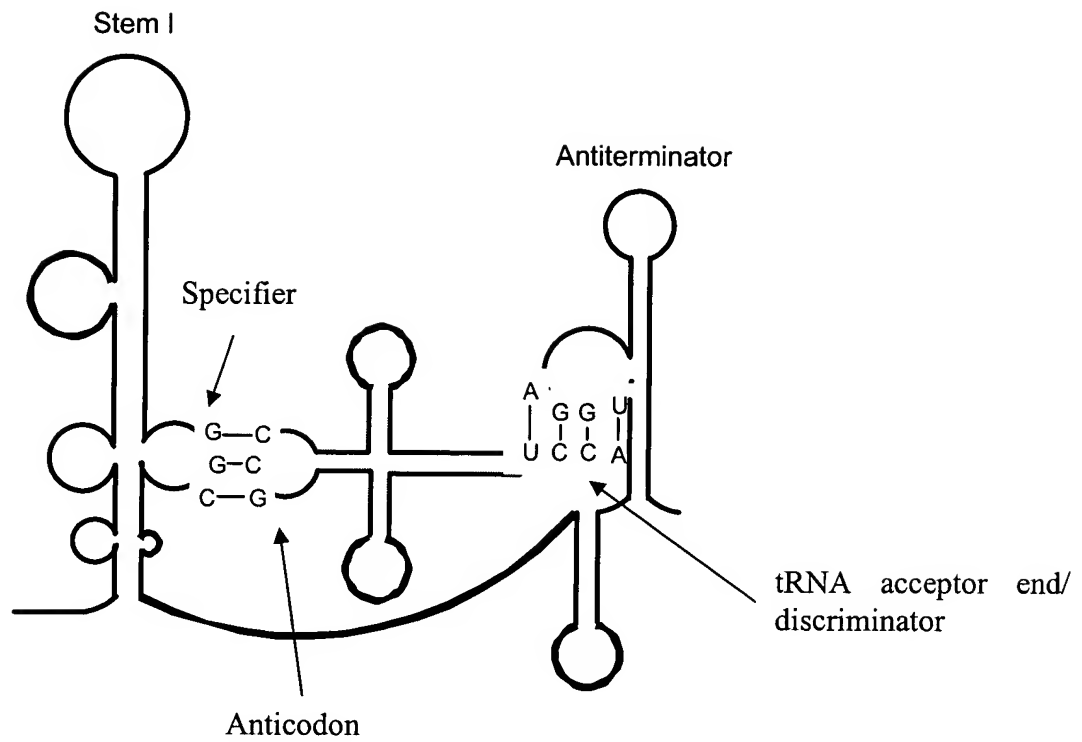


FIG. 7

5

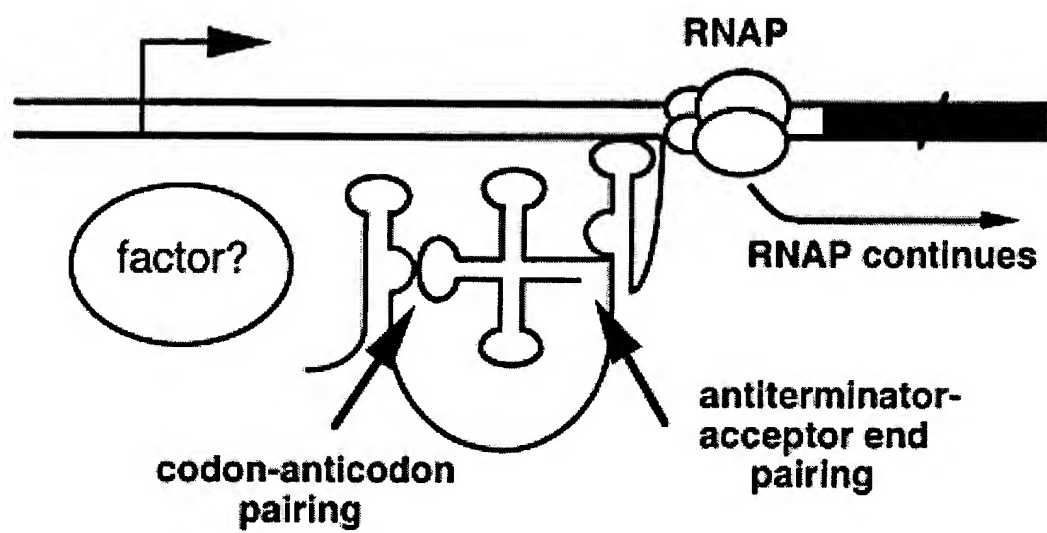


FIG. 8

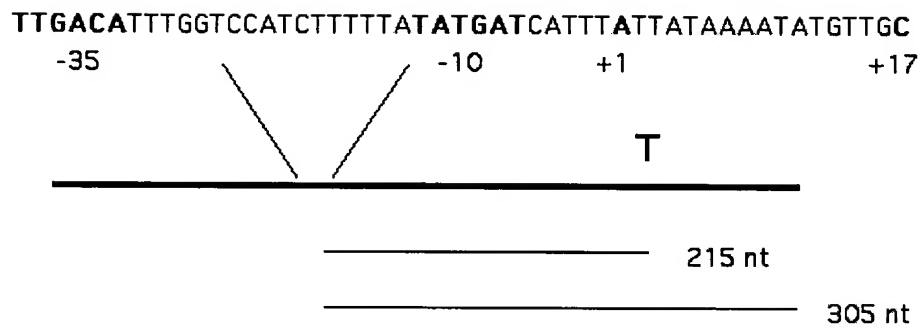


FIG. 9

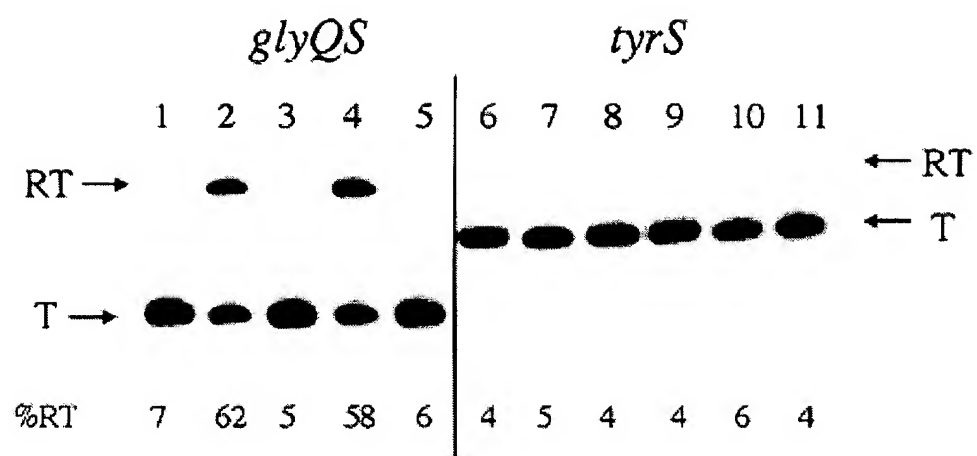


FIG. 10

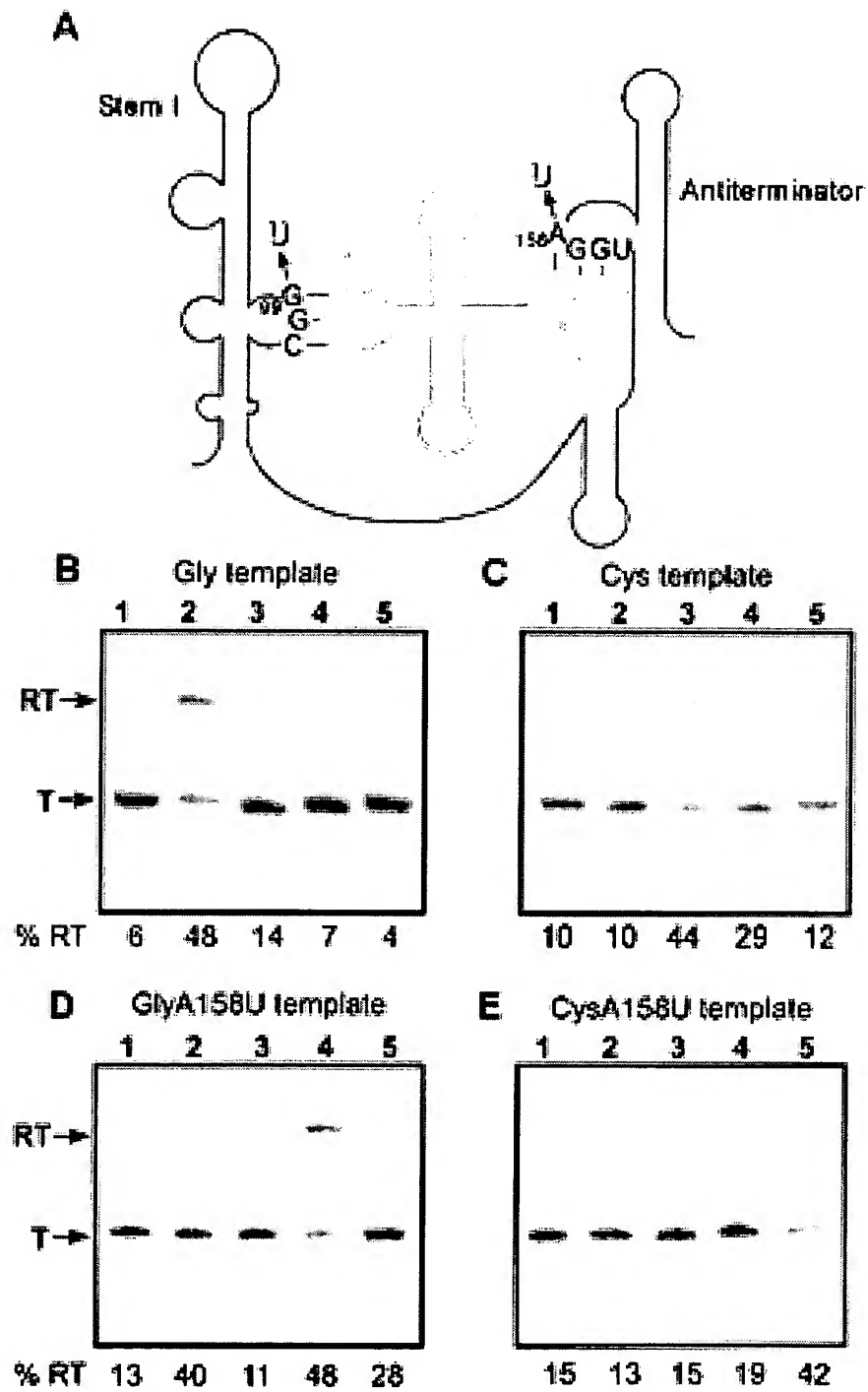


FIG. 11

Polynucleotide sequence for the *glyQS* gene from *Bacillus subtilis* corresponding to the *in vitro* transcription template: from *B. subtilis* 168.

```
ATTGATTTATATTACGAAGAATATTCGGGATTGTATTTAAATCAAAGCGCTTTTAGATCAAATGGAAAGCATGAA
ACATCTTATGGGTGAAAACAAAAGTTGACATTTGGTCCATCTTTTATATGATCATTATTATTAAATATGTTGCAG
TGAGAGAAAGAAGTACTTGCGTTTACCTCATGAAAGCGACCTTAGGGCGGTGTAAGCTAAGGATGAGCACGCAACGA
AAGGCATTCTTGAGCAATTTTAAAAAAGAGGCTGGGATTTTGTTCTCAGCAACTAGGGTGGAACCGCGGGAGAACTC
TCGTCCCTATGTTTGCGGCTGGCAAGCATAGAGACGGGAGTTTTTTGGTTGCTGCCGCAGTCAACTTATGAAAGAAA
AGTGGAGGTGCTTGAAATGAATATTCAAGACATGATTCTAACCTTGCAAAGC
```

FIG. 12

Sequence of same region from BR151MA (T to A at +6 position relative to transcription start-site)

```
ATTGATTTATATTACGAAGAATATTCGGGATTGTATTTAAATCAAAGCGCTTTTAGATCAAATGGAAAGCATGAA
ACATCTTATGGGTGAAAACAAAAGTTGACATTTGGTCCATCTTTTATATGATCATTATTTATAAAATATGTTGCAG
TGAGAGAAAGAAGTACTTGCGTTTACCTCATGAAAGCGACCTTAGGGCGGTGTAAGCTAAGGATGAGCACGCAACGA
AAGGCATTCTTGAGCAATTTTAAAAAAGAGGCTGGGATTTTGTTCAGCAACTAGGGTGGAACCGCGGGAGAACTC
TCGTCCCTATGTTTGCGGCTGGCAAGCATAGAGACGGGAGTTTTTGGTTGCTGCCGCAGTCAACTTATGAAAGAAA
AGTGGAGGTGCTTGAAATGAATATTCAAGACATGATTCTAACCTTGCAAAGC
```

FIG. 13

tRNA^{Gly} DNA sequence: (from SubtiList, confirmed by sequencing of region in BR151MA)

GCGGAAGTAGTTCAGTGGTAGAACACCACCTTGCCAAGGTGGGGGTCGCGGGTTCGAATCCCGTCTTCGCTCCA

FIG. 14

PCR primers used for preparing *glyQS* template:

GlyQUS1Xba: ATTGATCTAGATTACGAAGAATATTCGGGATTGTA (contains two changes from sequence shown in Fig. 12 to introduce an XbaI site (TCTAGA) at the 5' end of the fragment for generation of the *glyQS-lacZ* fusion construct)

GlyQDS2H3Pac: GGGTATTTAATTAAGCTTTTGCAAGGTTAGAATCA (introduces extra 14 nt downstream of *glyQS* sequence shown in Fig. 12 to provide a HindIII site (AAGCTT) for generation of the *glyQS-lacZ* fusion construct)

The PCR primers used to generate the *tyrS* template:

TyrUSBM: GGCTGGGGATCCGTCAACAATGGAGG

TyrN2b: CCGCGGAAGGATAAAGCTTCAAGTAAG

FIG. 15

Polynucleotide sequence for the *tyrS* template DNA (identical for 168 or BR151MA).

```
GGCTGGAGATCTGTCAACAATGGAGGATTAAGGCGGCGTTGACACAGGATTTTATTTATGTTAAAAATGATATAG
CTTCATATGAAAAGGTAAAGATTGAGACAAGTAGAATATCCTTACGTTCCAGAGAGCTGATGGCCGGTGAAAATCAG
CACAGACGGATATATCGAATACACTCATGAACCGCTTTTGCAAACAAAGCCGGCCAGGCTTTCAGTAGTGAAAGAAC
GGACCTGATCCGTTATCAGGCAAAGTGATAAGACGAATGTTTGCATTCTCTTATTAGTAGGGTGGTACCGCGATAAT
CAATCGTCCCTTCGTGTAAACGAAGGGGCGTTTTTTATTTTAATTAAAAAAGGAGCTTTATCTTATGACTAATTAC
TTGAAGACTTATCCTTCCGCGG
```

FIG. 16

The oligos used to generate tRNA^{Tyr}:

5': TyrUSBM: GGCTGGGGATCCGTCAACAATGGAGG
3': TyrN2b: CCGCGGAAGGATAAAGCTTCAAGTAAG

5': tRNATyrUST7: TAATACGACTCACTATAGGAGGGGTAGCG (the tRNA sequence starts with GGAGGGGTAGCG)
3': tRNATyrDS: TGGTGGAGGGGGGCAGATTCTG

The oligos used to generate tRNA^{Gly}:

5': GlytRNAUS: TAATACGACTCACTATAGCGGAAGTAGTTCAGTGG
(the first part of this oligo is the T7 promoter; the tRNA starts with GCGGAAGTAGTTCAGTGG)
3': GlytRNADS: TGGAGCGGAAGACGGGATTCTGAAC

FIG. 17

tRNA^{Tyr} DNA sequence.

GGAGGGGTAGCGAAGTGGCTAAACGCGGCGGACTGTAAATCCGCTCCCTCAGGGTTCGGCAGTTCGAATCTGCCCCC
CTCCACCA